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QUANTITATIVE THIN-LAYER CHROMATOGRAPHIC MEASUREMENT OF N-TRIFLUOROACETYLADRIAMYCIN-14-VALERATE (AD 32) AND TRIFLUOROACETYLADRIAMYCIN (AD 41) IN BLOOD AND TISSUES

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SUMMARY

A thin-layer chromatographic method has been developed for the detection and measurement of N-trifluoroacetyladriamycin-14-valerate (AD 32) and its major metabolite trifluoroacetyladriamycin (AD 41). The procedure gives satisfactory linearity over a large range of concentrations. The coefficient of variability is about 10% over the entire range of usable concentrations, giving good reproducibility; sensitivity is 25 ng for both AD 32 and AD 41. Analysis is specific for AD 32 and AD 41 since adriamycin or more polar metabolites can be differentiated. Recovery is high (85—90%) and the method is simple and economical to use. Pharmacokinetics of AD 32 and AD 41 are reported in blood and some tissues of mice bearing Lewis Lung carcinoma.

INTRODUCTION

N-Trifluoroacetyladriamycin-14-valerate (AD 32) is a recent derivative of adriamycin [1—3] reported to display greater antitumoral activity in animals than the parent compound. Recently it was introduced in a (clinical trial) phase I study. The chemicophysical and biological characteristics of this compound are completely different from those of adriamycin, and its mechanism of action is thus still unclear. For instance, it is completely insoluble in water, does not intercalate with DNA [4, 5] and the mode of its cytotoxic action [6, 7] appears to differ, at least in part, from that of adriamycin.

The ester bond at position 14 formed by adriamycin and valeric acid is easily split by blood and tissue esterases, giving rise to the metabolite N-trifluoroacetyladriamycin (AD 41), (Fig. 1), whereas the amide bond between the amino group on the glycosidic moiety and trifluoroacetic acid seems relatively stable [8]. AD 41 has been reported to maintain the inability of AD 32 to in-

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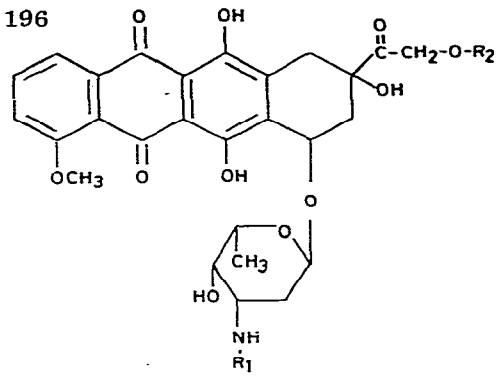


	R_1	R_2
Adriamycin	H	H
AD 32	$COCF_3$	COC_4H_9
AD 41	$COCF_3$	H

Fig. 1. Structures of adriamycin, N-trifluoroacetyl-adriamycin-14-valerate (AD 32) and N-trifluoroacetyl-adriamycin (AD 41).

tercalate with DNA but it is cytotoxic [5] and shows antitumoral activity in vivo [9].

The metabolism of AD 32 was studied in detail by Israel and co-workers [10, 11]. They found AD 41 in blood, urine and bile together with more polar metabolites such as 13-dihydro-N-trifluoroacetyladriamycin-14-valerate (AD 60) and N-trifluoroacetyladriamycinol (AD 92) and traces of adriamycin and adriamycinone. AD 41 was always the major metabolite. So far no data are available on the tissue distribution of AD 32 and its metabolites.

In this paper we present a simple method of measuring levels of AD 32 and AD 41 routinely in blood and tissues. This method is less senstive than that presented by Israel et al. [11], based on thin-layer chromatographic (TLC) separation followed by high-pressure liquid chromatographic analysis, but is less time-consuming and permits a large number of analyses to made easily, which is an important prerequisite for accurate pharmacokinetic studies. Furthermore, since a sufficiently specific assay of adriamycin and its derivatives in tissues is always a serious analytical problem, the method described in this paper may be of interest for the study of the pharmacological effects of AD 32 and its metabolites.

MATERIALS AND METHODS

Chemicals

All solvents were reagent grade (Carlo Erba, Milan, Italy). Silica gel plates 20 × 20 cm, 0.25 mm thick, without fluorescence indicator and without calcium sulphate binder were purchased from Merck, Darmstadt, G.F.R. N-Trifluoroacetyladriamycin-14-valerate (AD 32), N-trifluoroacetyladriamycin (AD 41), adriamycin, adriamycinol and daunomycinone were kindly supplied by Farmitalia, Milan, Italy. AD 32 and AD 41 were dissolved at a concentration of 6 mg/ml in saline containing 10% Tween 80. N-Trifluoroacetyladriamycinol (AD 92) was a kind gift of Dr. Mervyn Israel, Sidney Farber Medical School, Boston, Mass., U.S.A.

TLC scanning

Fluorescence scans were made with a Turner 111 TLC scanner, at an excitation wavelength of 475 nm and emission at 580 nm, connected with a Kontron W + W 1001 recorder.

Animals

C57B1/6 male mice (20 ± 2 g body weight), obtained from Charles River Italy (Calco, Italy), were used for these experiments. The animals received an intramuscular transplant of 2×10^5 viable cells of the Lewis Lung carcinoma, maintained by passages in the same strain every two weeks.

AD 32 was administered intravenously at a dose of 80 mg/kg 14 days after tumor transplantation. Animals were killed at various intervals of time after drug administration, and blood, heart, tumor and spleen were immediately collected. Tissues were repeatedly washed in chilled saline to eliminate blood contamination. Four animals were used each time.

Extraction procedure

Blood was collected in heparinized tubes and immediately cooled to 0°; 0.5 ml of blood was then mixed with 0.8 ml chilled water and deproteinized by the addition of 0.2 ml of cold acetone. These operations normally took less than 2 min. Table I gives a comparison of AD 32 hydrolysis at 0°, 25° and 37° by blood esterases. Within the first 2 min at 0° and 25° less than 10% of the drug is hydrolysed, whereas at 37° during the same period 45% of the compound is hydrolysed. The samples were extracted twice with 3 ml of diethyl ether, and the ethereal phases pooled and taken to dryness under a gentle stream of nitrogen. Frozen tissues were homogenized with an Ultra Turrax apparatus, Model TP 18/2N (Jane and Kungel, Staufen, G.F.R.) in chilled water in ratios of 1:4 or 1:10 (g/ml), and 0.2 ml of cold acetone was added to 0.5—1.0 ml samples. The samples were extracted twice with 3 ml diethyl ether and taken to dryness as described above.

TABLE I

HYDROLYSIS OF AD 32 BY BLOOD

Ten micrograms of AD 32 were added to 1 ml of control C57B1/6 mice blood. The mixture was incubated at different temperatures for 2—30 min and AD 32 and AD 41 were measured as described in the text. Each value is the mean of three determinations.

Incubation time (min)	Hydrolysis (%)		
	0°	25°	37°
2	8.0	6.0	45
5	8.9	13.0	49
10	9.7	38.0	48
30	10.0	72.0	62

Sample spotting

The residues were dissolved in 100—200 μl of an acetone solution of daunomycinone (20 μg/ml) used as standard; 5 μl were spotted on a silica gel plate

using a micropipette (Camag). On each plate eight unknown samples were spotted with four internal standards obtained by adding 0.05—2 μg of AD 32 and AD 41 to blood or tissue from control animals, and extracting as described for unknown samples. The plates were developed to 15 cm from the origin in a lined tank and were then removed and dried briefly before scanning.

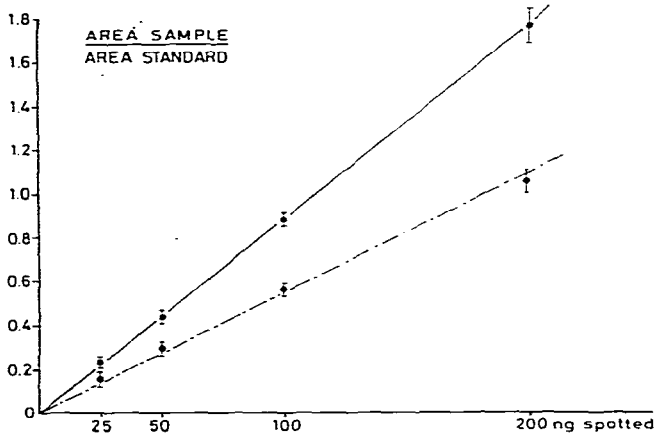
RESULTS AND DISCUSSION

Linearity and reproducibility

As shown in Fig. 2 the standard curve for this method was linear in the range 25—200 ng for AD 32 and AD 41. Each value is the mean ± S.E. of 15 different determinations. The reproducibility of the method was determined by scanning five times three different plates on which 25—200 ng AD 32 and AD 41 were spotted together with a known amount (100 ng) of daunomycinone as internal standard. The coefficient of variation for this analysis is 11.5 ± 2.2% for AD 32 and 9.3 ± 2.1% for AD 41.

Recovery

Recovery of AD 32 and AD 41 from blood and tissues was established by comparing the TLC peak areas for samples with AD 32 or AD 41 added with those on plates where the same amount of AD 32 and AD 41 had been spotted directly. Recovery was 90 ± 5% for blood and 85 ± 7% from tissues.



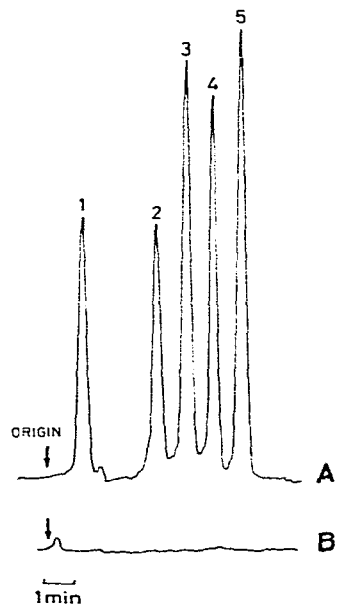


Fig. 2. Standard curves for AD 32 and AD 41. Mean and S.E. of 15 determinations are given at each concentration. ■, AD 32; ●, AD 41.

Fig. 3. (A) TLC scanning of 50 ng AD 92 (peak 1), 50 ng AD 41 (peak 2), 100 ng adriamycinone (peak 3), 75 ng AD 32 (peak 4) and 100 ng daunomycinone (peak 5) added as internal standard. (B) TLC scanning of a control blood sample.

Resolution and specificity

Development of the plates with the solvent system chloroform—methanol—acetic acid (93:5:2) gave the following R_F values: daunomycinone, 0.79; AD 32, 0.64; adriamycinone, 0.62; AD 41, 0.43; AD 92, 0.14 (Fig. 3A). Under the conditions employed adriamycin and adriamycinol do not move. Moreover, these two compounds are not recovered at all from blood and tissues. Thus the only possible interfering metabolites are the non-polar ones. Blood and tissues blanks did not give any interfering peaks during the TLC analysis (Fig. 3B).

Pharmacokinetics of AD 32

Fig. 4 shows the kinetics of AD 32 and AD 41 in blood after a single intravenous injection of AD 32 (80 mg/kg) in C57B1/6 mice bearing Lewis Lung carcinoma. AD 41 is detectable almost immediately after injection, suggesting that biotransformation of AD 32 into its major metabolite is very rapid. Their rates of disappearance from blood are quite different, AD 32 having a K_{el} of 0.20 (μg/ml), almost three times that of AD 41, suggesting that the metabolite is present in the blood compartment in higher amounts and for longer than its parent compound. Table II reports the peak levels of AD 32 and AD 41, the corresponding areas under the curve and half-lives ($T_{½}$) in blood and in the tumor, heart and spleen. Except in the blood, the AD 41 peak levels are always higher than AD 32 peaks; the areas under the curves 24 h after treatment and the half-lives indicate clearly that tumor, heart and spleen are much more exposed to AD 41 than to AD 32. The significance of these data deserves more studies, in particular to establish (a) if AD 32 is active per se or if it acts as a pro-drug, being hydrolysed by blood and tissue esterases, and (b) if the tissue distribution of AD 41 is simply dependent on the presence of esterases or depends also on its chemicophysical characteristics.

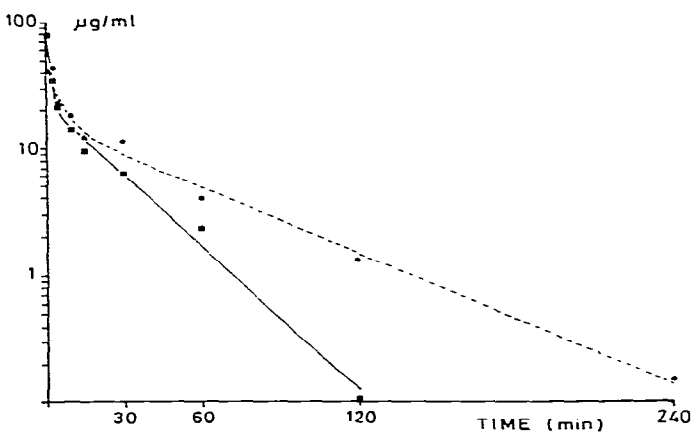


Fig. 4. Blood levels of AD 32 and AD 41 after intravenous injection of AD 32 (80 mg/kg) in C57B1/6 mice bearing Lewis Lung carcinoma. ▪———▪, AD 32; ●- - - - ●, AD 41.

TABLE II

BLOOD AND TISSUE LEVELS OF AD 32 AND AD 41 AFTER INTRAVENOUS ADMINISTRATION OF AD 32 (80 mg/kg) TO C57B1/6 MICE BEARING LEWIS LUNG CARCINOMA

Treatment was given on day 14 after tumor transplantation. In parentheses the time of peak concentration. The area under the concentration versus time curve (AUC) was measured by trapezoidal integration up to the last time at which the drug was detectable. The half-life ($T_{½}$) was assessed by calculating the significance of the regression using the determination coefficient r^2 (least-squares method).

	Peak level (μg/ml) or (g)		AUC after 24 h (μg/ml) or (g × min)		Half-life (min) or (h)	
	AD 32	AD 41	AD 32	AD 41	AD 32	AD 41
Blood	77.1 ± 2.4 (1 min)	44.8 ± 0.5 (3 min)	705 ± 65	990 ± 187	16 min	35 min
Tumor	5.2 ± 0.6 (10 min)	19.3 ± 1.5 (30 min)	161 ± 15	11,189 ± 1210	21 min	16 h
Heart	7.4 ± 2.6 (1 min)	87.0 ± 4.4 (1 min)	569 ± 55	7,002 ± 977	8 min	1.4 h
Spleen	11.8 ± 1.4 (1 min)	108 ± 5.7 (30 min)	72 ± 19	20,075 ± 1684	N.D.*	1.15 h

*N.D. = Pharmacokinetic analysis not possible because of insufficient data.

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